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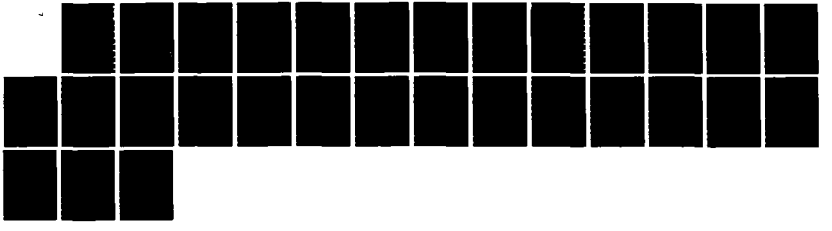

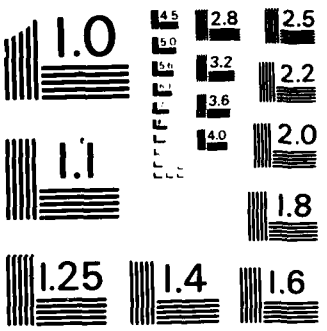

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REPORT NUMBER 2

# MODE OF ACTION OF POLYAMINE ANALOGUES ON THE GROWTH AND BIOCHEMISTRY OF LEISHMANIAL CELLS

Final Report

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The relationship between the growth of leishmanial parasites and polyamine biosynthesis was studied. Polyamines, mainly putrescine and spermidine, accumulated in macrophages infected with Leishmania tropica major promastigotes grown in vitro. Similar results were obtained, when tissues of BALB/C mice infected with L. tropica major were examined. A consistent increase in cellular putrescine and spermidine levels was observed in infected skin and spleen. With the accumulation of putrescine, a concomitant increase in ornithine decarboxylase activity

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was detected in growing leishmanial promastigotes and in macrophages supporting the growth of leishmanial amastigotes. An increase in the activity of ornithine and of S-adenosyl-L-methionine decarboxylases was also observed in Leishmania-infected skin and spleen from BALB/C mice. On the other hand, a temporary increase in polyamine levels and in the activity of the polyamine biosynthetic decarboxylases was noticed in the skin and spleen of leishmania-infected $C_3H$ mice.



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## Table of Contents

Summary

1. In the present study we extended the findings summarized in Report No. 1 and showed the relationship between the growth of leishmanial parasites and polyamine biosynthesis. Three systems were employed: 1. Leishmanial promastigotes grown in liquid media. 2. Leishmanial amastigotes grown on cultured mouse macrophages. 3. Animal experiments using BALB/C or $C_3H$ mice.

2. Polyamines, mainly putrescine and spermidine, accumulated in macrophages infected with Leishmaina tropica, in vitro. Similar results were obtained when tissues of BALB/C mice, infected with L. tropica major, were examined. On the other hand, only a transient increase in polyamine levels was observed in tissues of $C_3H$ mice infected with L. tropica major. These changes paralleled with the occurrence of parasites in the infected tissues.

3. With the accumulation of putrescine, a concomitant increase in ornithine decarboxylase activity was detected in growing leishmanial promastigotes and in macrophages supporting the growth of leishmanial amastigotes. A similar increase in the activity of ornithine decarboxylase was also observed in leishmaina infected skin and spleen from BALB/C mice.

4. The activity of S-adenosyl-L-methionine decarboxylase was also elevated in the skin and spleen of leishmania-infected BALB/C mice.

Foreword

In recent years biological research progressed significantly and many biochemical and genetic problems have been clarified. Thus, important new information has been gained on the biochemical properties of pathogenic bacteria as well as of viruses which cause diseases of humans.

Unfortunately, little progress has been made in the study of parasitic diseases and many of the biochemical problems related to host-parasite relationships have not been elucidated. Among the most important diseases of humans are those which are caused by intracellular parasites of reticuloendothenial cells; such diseases afflict tens of millions of people. Leishmania, a homoflagellate protozoan, causes leishmaniasis. The disease has a prevelence of at least 12 million parasitic infections in human and it poses a special problem in the Middle East, Africa, India, China, Central and South America (1). It represents

an immense public health problem to people living in the infected areas and also endangers businessmen, and tourists who travel to endemic regions. United States involvement in endemic areas in the last few years has not only meant technical assistance (Saudi Arabia, Central America), construction (Israel) manoeuvers (Egypt, Israel), operation (Iran) but also training in jungle warfare (Panama).

The lack of sufficient information on the biochemical properties of parasites has hampered the development of new drugs and indeed no revolutionary new therapeutic approaches have been advanced during the past three decades.

During the past twenty years, we have been studing the function of the naturally occurring polyamines, putrescine, spermidine and spermine (see sturcute in Fig. 1) and the regulation of their biosynthesis (Fig. 1). It is now generally accepted that these polycations regulate many growth processes (2-5). Thus increased concentrations of polyamines were first observed during chick embryo development (6) and in rat liver after partial hepatectomy (7). Subsequent reports describe the accumulation of polyamines in neoplastic tissues (8) and in cells transformed by tumor viruses (9) or treated with tumor promoting agents (8). Infection of cells with viruses, like Herpes simplex or Semliki Forest viruses also involves enhanced polyamine biosynthesis (10). It is conceivable that polyamines also accumulate in parasite-infected cells, when macromolecular synthesis increases.

Study of the structure of antiparasitic agents shows that a number are either alkylamine derivatives of polycyclic compounds or drugs resembling diamines and polyamines (Fig. 2); and it is thought that this similarity explaines their mode of action. Moreover, many antiparasitic drugs are of a cationic nature and structurally similar to polyamines. The activity of some of them is counteracted by exogenous polyamines. Thus, Newton (11) found that quinapyramine released putrescine from crithidial ribosomal preparations; and Wallis (12) noted that pentamidines release $Mg^{++}$ and polyamines from various trypanosomatid ribosomal preparations. Spermidine and spermine prevent bacteriostasis by propamidine (13). Ethidium also resembles polyamines and can replace spermidine in tRNA molecules

(14). Studies by Bacchi indicated that the curative effects of amicarbalide and imidocarb on Trypanosoma brucei-infected mice (15) was prevented by polyamines.

We have previously demonstrated the occurrence of the polyamines putrescine, spermidine and spermine in the various leishmanial promastigotes (16) and also reported the correlation between polyamine levels and the growth rates of the promastigotes (17). These findings prompted us to test compounds which inhibit polyamine biosynthesis for their antiparasitic activity. At least two of these compounds, namely MGBG (methylglyoxal bis-(guanylhydrazone) and sinefungin gave promising results (18,19). In addition, known antileishmanial agents were shown to inhibit the synthesis of polyamines (18). These findings supported our hypothesis that polyamines regulate the growth of leishmania sp. in analogy to its effect on other system. Independently, Bacchi et al (20) reported that trypanosomes and plasmodia contain polyamines. Moreover, they found that DFMO (α-difluoro-methylornithine), which inhibits polyamine biosynthesis, inhibits the growth of Trypanosoma brucei (21), Eimeria tenella and plasmodium falciparum (22). These findings suggest that polyamines regulate the growth of other parasites and that inhibition of their synthesis (or binding to cellular constituents) results in inhibition of growth.

During the past 6 months we extended our previous studies and focused our attention on the following points:

1. Comparison of the activity of ornithine decarboxylase activity and the growth rate of leishmanial promastigotes and amastigotes.
2. Determination of polyamine levels in the tissues of leishmania-infected animals.
3. Determination of the activity of polyamine biosynthetic decarboxylases (ODC, ornithine decarboxylase and SAMD, S-adenosyl-L-methionine decarboxylase) in the tissues of leishmania-infected animals.

List of illustrations and appendixes



Appendixes

Abstracts of papers presented at Annual Meeting of the Israel Biochemical Society and published in Israel J. Med. Sci.

## Materials and Methods

Organisms and media. The Leishmania tropica major strain (LRC-L137) was obtained from the culture collection of the World Health Organization's International Reference Center for Leishmaniasis (WHO-LRC), which is maintained in the Department of Protozoology at the Hebrew University-Hadassah Medical School, Jerusalem. The strain was maintained in vitro at 25-28°C, as promastigotes, by biweekly passage on either semisolid Locke-blood-agar medium (23) or diphasic N.N.N. medium (24). For experimental studies, promastigotes were grown at 25-28°C in a fully liquid, panmede-based medium that contained the following ingredients per 550 ml:1 g Panmede (Paines and Bryne Ltd., Pabyrne Laboratories, Greenford, Middlesex, England), 1 g glucose, 500 ml 0.9% sodium chloride, 10 ml 1.15% potassium chloride, and 40 ml normal rabbit serum, the final pH being adjusted to 8 to 8.2, and the medium being sterilized by Seitz filtration (25). This medium also served as the incubation medium in studies of comparatively short duration, i.e., hours rather than days. Living parasites were counted in hemocytometer.

Amastigotes were grown form promastigotes grown from a recent isolate of Leishmania tropica major LRC-L137 from Syrian hamsters, the promastigotes being put into cultures of $C_3H$ mouse macrophages that had been removed from mice, which had been stimulated with thioglycollate 5 days earlier. The ratio of promastigotes to macrophages was 2:1. Twenty hours after infection and transformation of the promastigotes to amastigotes, the culture medium was replaced by fresh McCoy's medium. Macrophages were checked daily for 4 days. Coverslips with infected macrophages were washed with phosphate buffered saline, fixed with methanol, stained with Giemsa's stain and mounted on glass slides.

Animal experiments. In conducting this research we adhered to the "Guide for the Care and Use of Laboratory Animals" prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council.

3 Week-old male BALB/C or $C_3H$ mice were injected intradermally at the base of the tail (plucked free of hair) with $3x10^6$ in vitro derived promastigotes of L. tropica, in 0.05 to 0.1 ml of normal saline. The state of the lession was assessed weekly. Finally, animals were sacrificed and polyamine levels and ornithine decarboxylase activity was determined in various tissues.

Analytical methods. Polyamines were extracted from parasites or tissues with 3% perchloric acid. Their dansyl-derivatives, prepared according to the method of Seiler (26), were separated and identified by thin-layer chromatography on 300µM-thick silica gel G plates, using ethyl acetate: cyclohexane (2:3) as the solvent. Quantification of the various polyamines was done fluorometerically. Once identified, dansyl-polyamines were scraped off plates, extracted with 4 ml quantitites of toluene and assayed in a Turner Model III Fluorometer (<exc. -365nm, emission -505nm), the fluorescence corresponding to the scraped spots being compared that of known standards. The activity of ornithine decarboxylase (ODC, L-ornithine carboxy-lyase EC 4.1.1.17) was determined by suspending cells or tissues in 1.0-ml quantities of EDTA buffer (10mM sodium phosphate buffer, pH 7.2, containing 0.1mM EDTA, 3mM dithiothreitol (Sigma Chemical Co., St. Louis, Mo); and 40µM pyridoxal-5-phosphate), and disrupting them by freezing and thawing twice. The supernatant fluid obtained after centrifugation at 4,000 g at 4°C was used for the estimation of decarboxylase activity. Tissues were treated similarly, except that they were extracted by homogenization. Enzyme activity was determined by measuring the release of $[^{14}C]O_2$ from $[^{14}C]$-ornithine. Reaction mixtures consisted of 0.25ml of cell extract an- 0.4µCi of $[^{14}C]$-ornithine (specific activity 51.3 mCi/mmol (New England Nuclear, Boston Mass, USA) in EDTA buffer at a final volume of 0.5ml. Incubations were carried out in 17x150mm glass tubes equipped with a rubber stopper supporting a polyethylene center well (Kontes Glass Co., Vineland, N.J., USA) that contained 0.2ml of hyamine hydroxide (Packard Co., Zurich, Switzerland). After shaking at 37°C for 60min, the reactions were terminated by injecting 0.2ml of 3% perchloric acid through the rubber stopper. The mixtures were agitated for an additional 20min at 37°C to ensure complete adsorption of the $[^{14}C]O_2$.

The center wells were then removed and their radioactivity counted as previously described elsewhere (27).

The active os S-adenosyl-L-methionine decarboxylase (SAMD, EC 4.1.1.15) was similarly determined except that 0.4µCi of $[^{14}C]$ S-adenosyl-L-methionine (specific activity 58µCi/µmole, Radiochemical Centre, Amersham, Bucks, England) and 1mM putrescine were present in the incubation mixture.

## Results

### 1. Changes in the activity of ornithine decarboxylases during promastigote growth

We previously demonstrated the presence of polyamines in leishmanial promastigotes (16) and showed the conversion of [$^{14}$C] putrescine into [$^{14}$C] spermidine and [$^{14}$C] spermine during parasite growth (16,18). Evidence was also presented showing the cellular polyamines levels fluctuate during the growth of leishmanial promastigotes in liquid medium, and that the molar putrescine/spermidine ratios correlate well with the derivative of the growth rate (17).

Preliminary experiments indicated that leishmanial promastigotes of strain LRC-L137 are capable of converting [$^{14}$C] ornithine into [$^{14}$C] putrescine. The following experiment was designed to see if leishmanial promastigotes exhibit ornithine decarboxylase activity, and if such activity is related to their cell cycle.

L. tropica major promastigotes were incoculated into Panmede m[illegible]um at 2.5x10$^6$ parasites/ml. At various times thereafter, parasites were co[illegible]d and 2-5ml quantities of the cultures were withdrawn. Promastigotes were [illegible]crifuged and 4-5x10$^7$ parasites were used for each assay of ornithine decarboxylase activity.

Figure 3 clearly shows the presence of ODC activity in leishmanial promastigotes. Moreover, a significant increase in enzyme activity was observed during the first 70 hours of growth, when the growth rate is maximal. A sharp decline in ODC activity was noticed 90 hours after the initiation of growth, 30 hours prior to decrease in the growth rate.

### 2. Polyamines and ornithine decarboxylase activity during amastigote growth

After showing that polyamines are significant in the growth of leishmanial promastigotes, their role in the growth of leishmanial amastigotes was studied. Mouse macrophages were infected with promastigotes of L. tropica major and polyamine levels of the infected cells were determined during the various stages of the growth and development of leishmanial amastigotes. It may be seen that putrescine accumulated in the infected macrophages (Table 1). The difference seen between normal and infected cells was most significant during the first day of amastigote development. After infection, a moderate increase in cellular sperimdine levels in the macrophages was also noticed. Spermine levels, on the other hand, were lower in the infected macrophages, compared to the normal controls (Table 1). The increase in cellular putrescine levels during the growth of leishmanial amastigotes might easily be explained by ODC activation. To test this, mouse macrophages were infected with L. tropica major parasites and the ODC activity was determined at various time intervals. It can be seen

(Fig. 4) that infected and non-infected macrophages exhibited ODC activity soon after seeding. This may be due to the activation of ODC by components present in growth medium e.g., serum, or a result of their previous history. It should be remembered that the macrophages were obtained after intraperitoneal injection of thioglycollate. This treatment might have affeced cellular ODC activity (28). Figure 4 also shows that the activity of ODC in the uninfected macrophages declines rapidly and reaches steady low plateau 20 hours after seeding. The behaviour of parasite-infected macrophages was significantly different and a consistent increase in ODC activity was observed 3 days after infection (Fig. 4).

3. Polyamines and the activity of ornithine and S-adenosyl-L-methionine decarboxylases during the growth of parasites in experimental animals

(a) Infected skin

After showing that the growth of leishmanial promastigotes and amastigotes is related to changes in polyamine levels, it was of interest to see whether similar events also take place in Leishmania-infected tissues. BALB/C mice were infected with L. tropica major and the polyamine levels in normal and infected skin were compared. It is evident from Fig. 5 that putrescine accumulates in the infected skin by one week after parasite injection. The changes in spermidine levels are even more dramatic, and a 2-3 fold increase was observed in the infected skin 1-2 weeks after injecting the parasites (Fig. 6).

It has been well established that the course of cutaneous leishmaniasis, differs markedly among various common inbred mouse strains. In BALB/C mice the infection in characterized by an expanding ulcerous lesion, while in $C_3H$ mice lesions resolve within 8 weeks (29). It was therefore of interest to find out whether polyamine levels also increase in the skin of $C_3H$ mice, infected with L. tropica. It may be seen (Fig. 7) that putrescine did not accumulate in the skin of infected $C_3H$ mice, unlike BALB/C mice in which a significant increase in putrescine level was observed after infection with L. tropica (cf Fig.5). The increase in spermidine levels in leishmania-infected skin of $C_3H$ mice was also moderate, mainly during the first two weeks (Fig. 7). In this respect $C_3H$ infected skin also differed from that of BALB/C mice.

The accumulation of polyamines in the skin of leishmania-infected mice is best explained by the increase in the activity of ornithine decarboxylase. Table 2 shows that normal skin of BALB/C mice did not show significant ornithine decarboxylase activity. Infection with L. tropica, triggered the activation of this enzyme in all the skin samples tested (Table 2).

(b) Infected spleen

Parasitic infection is not limited to the infected skin and parasites also multiply in other tissues containing elements of the lymphoid-myeloid comples such as the spleen of infected animals. It was not suprising to find that the infected spleen contains more polyamines than the normal tissue. Table 3 clearly shows that the putrescine level in BALB/C-infected spleen is approximately 50% higher than in the uninfected controls. Changes in spermidine and spermine levels were also noticed (Table 3). The picture was entirely different when spleens of $C_3H$ mice were analyzed. In these animals only a transient parasitemia was noticed and no parasites were detected in the spleen, 3 weeks after infection. It may be seen (Table 3) that polyamines accumulated in the spleen of $C_3H$ mice only during the first week after infection with L. tropica. Thereafter, the levels of the polyamines returned to the control values (Table 3).

Elevated polyamine levels in the Leishmania-infected spleen is apparantly due to the activation of ornithine decarboxylase. It is evident from Table 3 that the activity of ornithine decarboxylase increased more than 5-fold in BALB/C infected spleens, compared with the normal control. On the other hand, in infected $C_3H$ mice only a transient increase in ornithine decarboxylase was noticed in the infected spleen (Table 3). These changes paralleled the increase on cellular polyamine levels.

In addition to the increase in ornithine decarboxylase, infection with L. tropica also led to the activation of S-adenosyl-L-methionine decarboxylase in the spleen. Again, a significant increase in enzyme activity was observed in the spleen of BALB/C mice one and 3 weeks after infection (Table 3). On the other hand, only a temporary increase in S-adenosyl-L-methionine decarboxylase activity was observed in the spleen of $C_3H$ mice infected with L. tropica (Table 3).

## Discussion

It has been some 30 years since a truly novel drugs for the treatment of parasitic diseases was introduced. Existing drugs are toxic and drug resistance is spreading (30). A bett : understanding of the biochemistry of the parasites and the elucidation of the mode of action of known drugs are prerequisites for the development of new drugs. It would be ideal to detect qualitative or quantitative differences in the activity of some enzymes in normal and infected tissues. These differences may then be exploited for the development of specific or preferential therapeutic agents (31). In this study we observed such changes and consistent elevation of polyamines and their biosynthetic enzymes (ornithine and S-adenosyl-L-methionine decarboxylases) have been detected in the leishmania-infected tissues. Work is now in progress to ascertain that these changes, are reversible and can be eliminated upon treatment.

In the mean time the occurrence of polyamines in various trypanosomatids has been confirmed by other investigators (30,32) who also employed compounds which inhibit polyamine biosynthes for therapeutic purposes (22,33,34). It is now generally accepted that trypanosomatids behave like other eukaryotes and that their growth is related and perhaps also dependent on polyamine biosynthesis (17,18,30,34). Should this be the case then the growth of these parasites could be arrested by:

(a) Inhibition of polyamine biosynthesis.
(b) Interference with the biological functions of polyamines.

In eukaryotes the synthesis of polyamines is catalyzed by two enzymes, ornithine decarboxylase and S-adenosyl-L-methionine decarboxylase which catalyze the synthesis of putrescine and spermidine, respectively (Fig. 1). α-Difluoromethylornithine (which is a irreversible inhibitor of ornithine decarboxylase) (DFMO) arrests the growth of Trypanosoma brucei, but unfortunately has no effect of Leishmania tropica. Studies carried out in our laboratory suggest that the drug is not taken up by L. tropica; but it is highly active when tested against crude ornithine decarboxylase extracted from leishmanial promastigotes. Unlike DFMO, sinefungin (another ornithine analog) is taken up by leishmanial promastigotes and amastigotes and inhibit their growth (19).

Of the compounds which inhibit S-adenosyl-L-methionine decarboxylase, MGBG was found to inhibit the growth of Leishmania tropica (18) and of Tryponosoma brucei (33).

These promising leads certainly warrant further studies and we would like to synthesize and test the compounds described in the Renewal Application Submitted last year.

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TABLE 1

Changes in polyamine levels in mouse macrophages infected with Leishmania tropica major LRC-L137

| Time (h) | Infection | Polyamines (nmol/mg protein) | | |
|---|---|---|---|---|
| | | Putrescine | Spermidine | Spermine |
| 0.25 | + | 82.8 | 17.2 | 17.8 |
| | - | 30.0 | 12.3 | 10.0 |
| 21.5 | + | 46.5 | 21.8 | 9.4 |
| | - | 8.7 | 12.0 | 10.0 |
| 42.0 | + | 21.1 | 22.2 | 10.6 |
| | - | 18.2 | 26.4 | 25.5 |
| 70.0 | + | 21.1 | 18.3 | 16.0 |
| | - | 18.2 | 17.3 | 27.0 |
| 90.0 | + | 14.3 | 12.9 | 5.7 |
| | - | 13.8 | 9.2 | 13.8 |

## TABLE 2

Ornithine decarboxylase activites in the skin of Leishmania-infected mice

| Animal | Time after infection (weeks) | Ornithine decarboxylase activity (nmol/mg protein/hr) |
|---|---|---|
| BALB/C - Infected | 1 | 18.0; 5.6; 7.8 |
| | 2 | 7.1; 9.0; 6.1 |
| | 3 | 16.4 |
| | 4 | 13.5; 14.6 |
| BALB/C - Control | 1 | 0 0 0 |
| | 2 | 0 0 0 |
| | 3 | 0 0 0 |
| | 4 | 0 0 0 |

Mice were infected with Leishmania tropica major LRC-L137. Every week three animals were sacrificed and enzyme activities were determined in the skin of the infected animals and in that of normal controls.

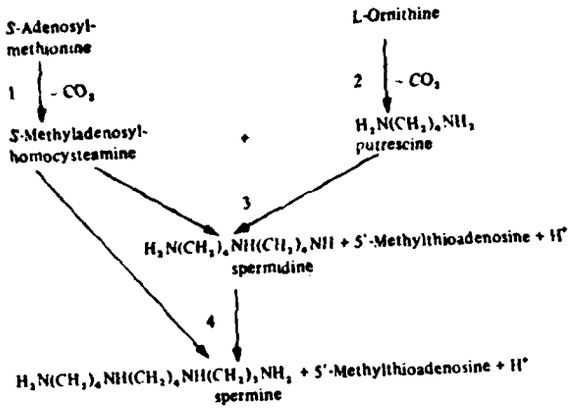
TABLE 3

Polyamine levels and decarboxylase activities in the spleen of normal and Leishmania-infected mice

| Animal | | Polyamines nmol/mg protein Putrescine | Spermidine | Spermine | Decarboxylase activity nmol/mg protein/hr ODC | SAMD |
|---|---|---|---|---|---|---|
| Balb/C - Infected | 1 | 2.3 | 21.3 | 15.3 | 6.0 | 62.9 |
| | 2 | 2.1 | 18.2 | 9.8 | 32.7 | -- |
| | 3 | 3.6 | 34.2 | 22.5 | 10.6 | 73.7 |
| Balb/C - Control | 1 | 1.7 | 12.0 | 8.1 | 1.13 | 47.6 |
| | 2 | 1.5 | 14.1 | 10.6 | 5.4 | -- |
| | 3 | 2.3 | 13.7 | 9.4 | 1.56 | 48.0 |
| $C_3H$ - Infected | 1 | 2.5 | 16.0 | 8.7 | 11.4 | 50.0 |
| | 2 | 1.5 | 9.5 | 6.2 | 0.7 | 21.9 |
| | 3 | 1.9 | 8.9 | 5.8 | 0.7 | 19.3 |
| $C_3H$ - Control | 1 | 1.6 | 6.1 | 4.8 | 0.56 | 24.0 |
| | 2 | 1.4 | 7.3 | 5.8 | 1.1 | 23.5 |
| | 3 | 1.2 | 5.4 | 4.0 | 1.3 | 26.0 |

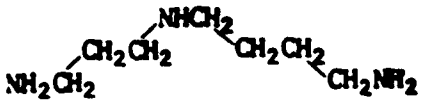
Samples were analyzed 4 weeks after infection.
Parasites were detected in the spleen.

ODC - Ornithine decarboxylase
SAMD - S-adenosyl-methionine decarboxylase

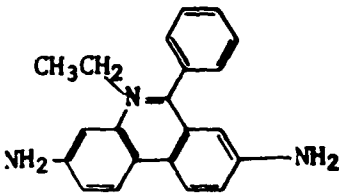



*Numeration:* 1, *S*-adenosyl-methionine decarboxylase; 2, ornithine decarboxylase; 3, aminopropyl transferase or spermidine synthase; 4, aminopropyl transferase or spermine synthase

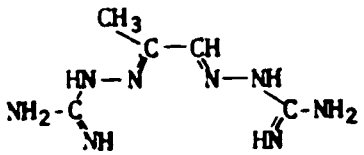
Fig. 1.



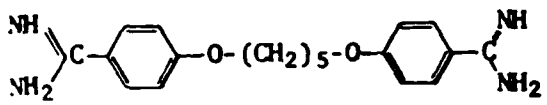
Spermidine



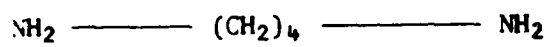
Ethidium



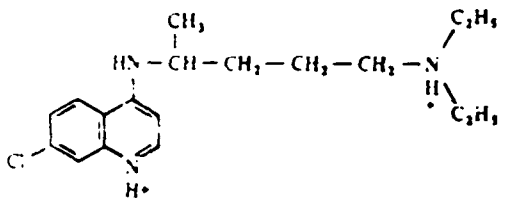
MGBG



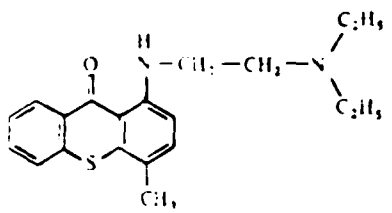
Pentamidine



Putrescine



Chloroquine



Miracil D

Fig. 2.

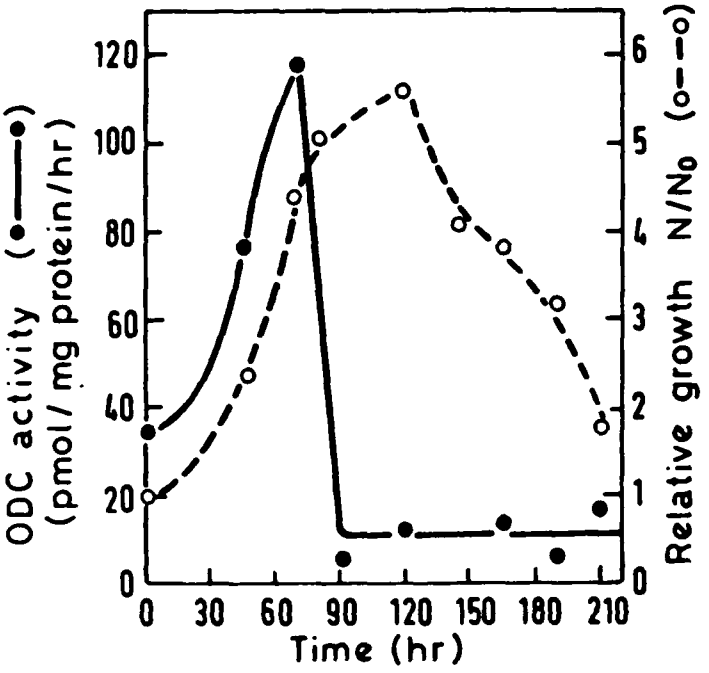

ODC activity (•——•)
(pmol/ mg protein/hr)
Relative growth N/N0 (o--o)
120
100
80
60
40
20
0
6
5
4
3
2
1
0
0 30 60 90 120 150 180 210
Time (hr)

Fig. 3.

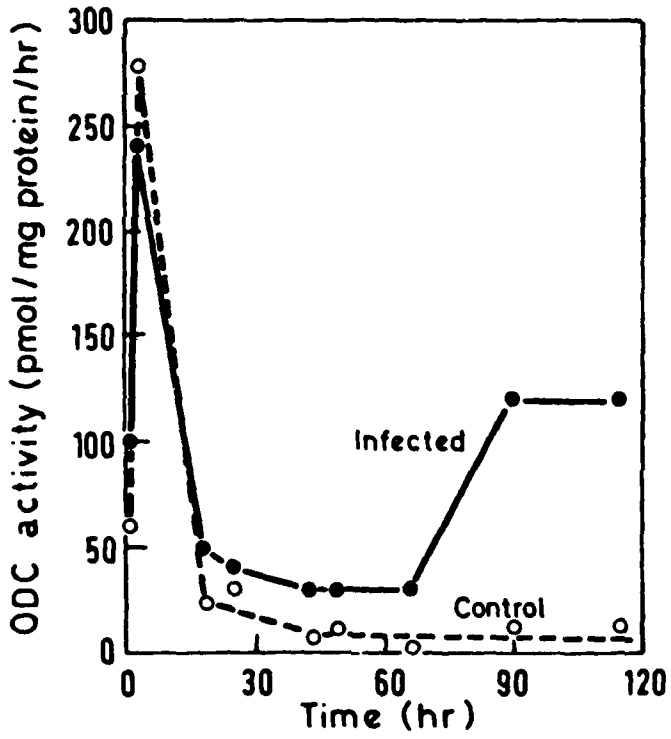
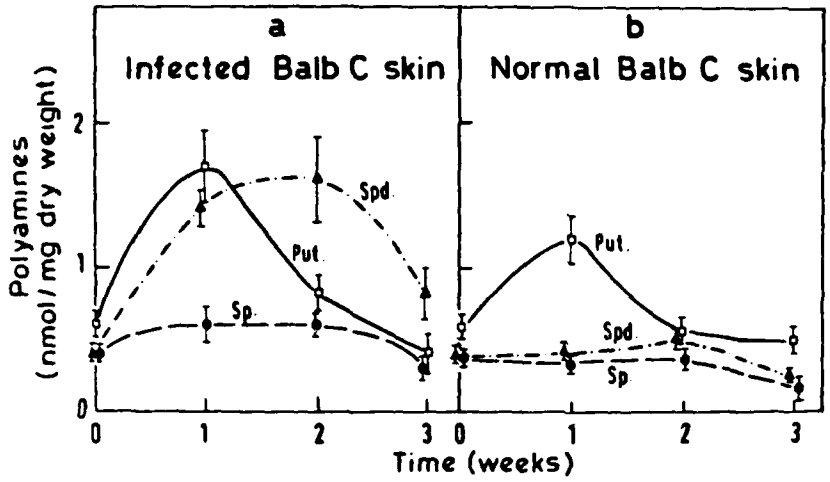

ODC activity (pmol/mg protein/hr)
300
250
200
150
100
50
0
0
30
60
90
120
Time (hr)
Infected
Control

Fig. 4.


a
Infected Balb C skin
b
Normal Balb C skin
Polyamines
(nmol/mg dry weight)
Put
Spd
Sp
Time (weeks)
0
1
2
3

Fi. 5.

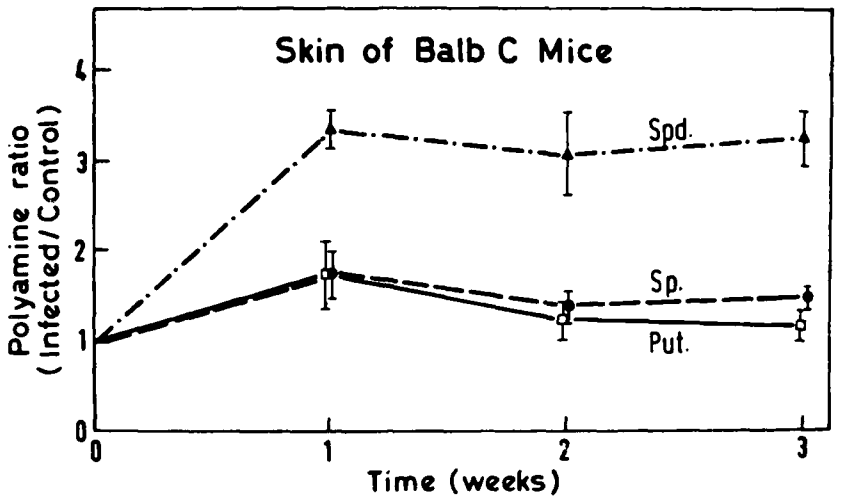

Skin of Balb C Mice
Polyamine ratio
(Infected/Control)
Spd.
Sp.
Put.
Time (weeks)
0
1
2
3
4

Fig. 6.

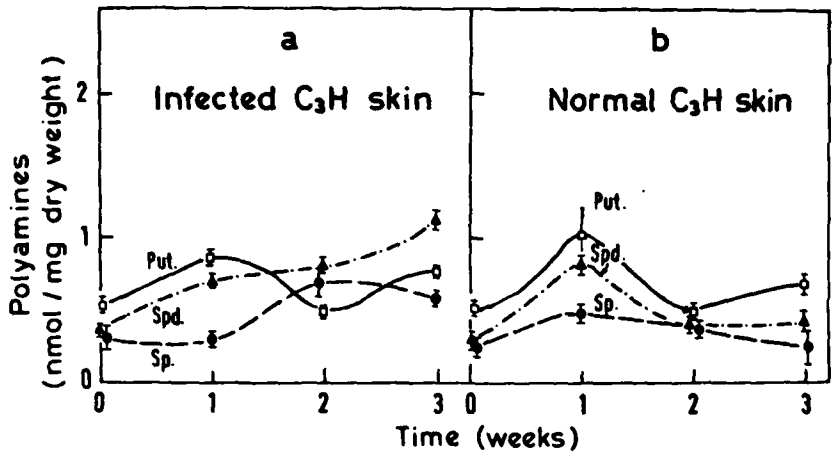

a
Infected C3H skin
b
Normal C3H skin
Polyamines
(nmol / mg dry weight)
Put.
Spd.
Sp.
Time (weeks)
0
1
2
3

Fig.7.

APPENDIX

PROCEEDINGS OF THE ANNUAL MEETING ISRAEL BIOCHEM SOC., JERUSALEM 1981

**Synthesis and content of polyamines in the tissues of mice infected with *Leishmania tropica*.** L. Abu-Elheiga, L. F. Schnur, J. El-On, C. L. Greenblatt and U. Bachrach, Hebrew University–Hadassah Medical School, Jerusalem.

We have previously shown that polyamine levels rise significantly in leishmanial promastigotes during growth. A similar increase was observed during the growth of leishmanial amastigotes in cultured mouse macrophages. To test the generality of these findings, animal experiments were carried out as follows: BALB/c and $C_3H$ mice were infected with *Leishmania tropica* and the polyamine content of various infected tissues was determined. A significant increase in cellular spermidine was observed in the skin of BALB c and $C_3H$ mice. The kinetics of the changes seen differed in the two mouse strains. In infected BALB/c mice, the increase in polyamines was more dramatic and was apparent as early as one week after infection. Similar changes in polyamine levels were also observed in infected liver and spleen from BALB/c and $C_3H$ mice. A concommitant and dramatic increase in the activity of ornithine decarboxylase and *S*-adenosyl-L-methionine (polyamine-biosynthetic decarboxylase) was also noticed in infected skin, liver and spleen. These findings stress the general importance of polyamines in leishmanial growth, extending our knowledge from *in vitro* to *in vivo* systems.

**Polyamine synthesis and levels in leishmanial amastigotes grown in mouse macrophage cultures.** M. Talmi, J. El-On, L. F. Schnur, C. L. Greenblatt and U. Bachrach, Hebrew University–Hadassah Medical School, Jerusalem.

The leishmaniases are cosmopolitan diseases caused by protozoan parasites that can also be grown *in vitro* either as free-living promastigotes or as intracellular amastigotes. Despite the high incidence and the overall severity of the diseases, therapy has remained fairly static for the last 30 years. No new drugs have been developed and little progress has been made in elucidating the mode of action of the drugs currently in use. We have shown that leishmanial promastigotes produce polyamines and that these are probably important in the parasite cell cycle. Here we report changes in the activity of ornithine decarboxylase (ODC), the rate-limiting enzyme in polyamine biosynthesis during growth of promastigotes. The activity of this enzyme also increased markedly in amastigotes grown in cultures of mouse peritoneal macrophages. A concommitant increase in the cellular levels of both putrescine and spermidine was also observed. The consistent finding of polyamines correlating with leishmanial growth may have practical implications in studying the mode of action of drugs and their further development.

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